Paper # **C-012**

A Multi-Site Study Comparing an 18-24h Sensititre® Susceptibility System to the CLSI Broth Microdilution Method for Oritavancin Using Fastidious and non-Fastidious Gram-positive Organisms

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ABSTRACT

Background: Oritavancin (ORI) (The Medicines Company, Parsippany, NJ), is a semi-synthetic lipoglycopeptide antibiotic with bactericidal activity against gram-positive organisms. An evaluation was performed to determine the accuracy and reproducibility of oritavancin susceptibility testing using the Sensititre® dried susceptibility system (TREK Diagnostic Systems, Cleveland, OH) compared with with the CLSI M07-A7 reference broth microdilution method (BMD). Both automated and manual reading methodologies were employed.
Materials and Methods: ORI (0.0005-8µg/mL) was tested against 855 recent clinical isolates, 150 challenge isolates and 50 reproducibility isolates. These isolates consisted of: 117 coagulase-negative *Staphylococcus* spp., 171 *Staphylococcus aureus*, 109 *Enterococcus* spp., 334 ß-hemolytic Streptococci, 190 *Streptococcus pneumoniae,* and 134 Viridans Group streptococci. Dried plates were inoculated per manufacturers' instructions. BMD was performed per CLSI M07-A7 guidelines. Recommended CLSI quality control (QC) organisms were tested daily and all results were within the CLSI published QC ranges.
Results: Comparisons of MIC results on the Sensititre® system to the CLSI M07-A7 BMD for automated reads using Sensititre ARIS® 2X and manual reads resulted in 96.2% and 98.3% essential agreement for ORI (+/- one $\log_2$ dilution), respectively. Overall agreement for the reproducibility (+/- 2 $\log_2$ dilution of the modal MIC) using automated and manual reads were 95% and 96%, respectively.
Conclusions: The results for ORI indicates that the Sensititre® susceptibility system for all clinical and challenge isolates gave reliable results using either the automated or manual read methods compared to the reference CLSI BMD.

INTRODUCTION

Oritavancin is a novel semi-synthetic lipoglycopeptide that is currently in late-stage clinical development for the treatment of serious gram-positive infections, including MRSA. Its activity in vitro is best described as rapidly bactericidal and its spectrum includes isolates that are resistant to currently-used antibiotics such as vancomycin, daptomycin and linezolid.

PURPOSE OF THE STUDY

A multi-site study to evaluate the performance of **oritavancin** on the Sensititre® 18 – 24 hour automated/manual susceptibility plate compared to the CLSI reference broth microdilution Method (M07).

MATERIALS & METHODS

Antimicrobial Tested	Range Tested	Supplied By
Oritavancin (ORI)	0.0005-8µg/ml	The Medicines Company, Parsippany, NJ

Organisms Tested	
Clinical Isolates (combined 3 sites) (394 gram-positive and 461 fastidious isolates)	855
CDC challenge isolates (one site) (75 gram-positive and 75 fastidious isolates)	150
Reproducibility isolates (combined 3 sites) (25 gram-positive and 25 fastidious isolates)	50
CLSI Quality Control Strains (20 replicates of each strain at three sites)	60

Clinical and Challenge Isolates Tested

Gram-Positive Species	Number Tested
Staphylococcus aureus	112
Coagulase Negative *Staphylococcus* spp.	162
Enterococcus spp.	103
Streptococcus spp. Beta-haemolytic Group	92
Total	**469**

Streptococcus Species	Number Tested
Streptococcus pneumoniae	180
Streptococcus spp.Viridans Group	129
Streptococcus spp. Beta-haemolytic Group	227
Total	**536**

QUALITY CONTROL

- Recommended CLSI quality control (QC) organisms were tested daily and were within the CLSI expected QC ranges.
- Colony counts were performed and fell within expected ranges.
  - CLSI: 2 - 8 x 10^5, Sensititre: 5 x 10^4 - 5 x 10^5 units of CFU/mL

Quality Control Strains	CLSI MIC Ranges (µg/mL)
Staphylococcus aureus ATCC 29213	0.015-0.12
Enterococcus faecalis ATCC 29212	0.008-0.03
Streptococcus pneumoniae ATCC 49619	0.001-0.004

SUSCEPTIBILITY TESTING METHODS

- Indications for use: The Sensititre 18-24 hour MIC or breakpoint susceptibility system is an *in vitro* diagnostic product for clinical susceptibility testing of both fastidious and non-fastidious organisms.
- Each isolate was tested using a Sensititre® 18 – 24 hour susceptibility plate containing **oritavancin**. The plates were set up and tested according to the manufacturers' instructions.
- The CLSI reference broth microdilution plate was prepared and tested on each isolate according to Clinical Laboratory and Standards Institute (CLSI M07) methods.
- Strains included 855 fresh clinical isolates; 132 gram-positive isolates, and 153 fastidious isolates from each site. 150 challenge isolates consited of: 75 gram positive and 75 fastidious supplied to a single testing site.
- Reproducibility testing consisted of 25 gram-positive and 25 fastidious isolates tested at all 3 sites on the Sensititre® 18-24 hour susceptibility plate. The test plate results were compared to those of the CLSI reference broth microdilution plate.
- Quality control was assured by testing 20 replicates of the ATCC strains, *S. aureus* ATCC 29213, *E. faecalis* ATCC 29212, *S. pneumoniae* ATCC 49619, at each site.
- Colony counts were performed on the inoculum of the QC strains on each day of testing.

RESULTS

Essential agreement for the clinical and challenge isolates were calculated for each method (Auto read and Manual) read using the +/-one $\log_2$ dilution standard. The essential agreement rates for the clinical and challenge isolates were as follows:

Summary Data and % Essential Agreement of *Streptococcus* species Clinical and Challenge Isolates using the Manual Read Method

Clinical Isolates

Organism Group	Total of all Isolates Tested	Essential Agreement of Total	% Essential Agreement of Total
Streptococcus pneumoniae	155	150	96.8%
Streptococcus spp. Viridans Group	104	98	94.2%
Streptococcus spp. Beta-Hemolytic Group	202	199	98.5%
Total	**461**	**447**	**97.0%**

Challenge Isolates

Organism Group	Total of all Isolates Tested	Essential Agreement	% Essential Agreement of Total
Streptococcus pneumoniae	25	25	100.0%
Streptococcus spp. Viridans Group	25	22	88.0%
Streptococcus spp. Beta-Hemolytic Group	25	25	100.0%
Total	**75**	**72**	**96.0%**

Total Isolates

Organism Group	Total of all Isolates Tested	Essential Agreement of Total	% Essential Agreement of Total
Streptococcus pneumoniae	180	175	98.4%
Streptococcus spp. Viridans Group	129	120	91.1%
Streptococcus spp. Beta-Hemolytic Group	227	224	99.3%
Total	**536**	**519**	**96.8%**

**Streptococcus* spp. Beta-hemolytic Group was tested with Mueller Hinton Broth supplemented with 2.5% Lysed Horse Blood

RESULTS cont.

Summary Data and % Essential Agreement of *Streptococcus* species Clinical and Challenge Isolates using the Auto Read Method

Clinical Isolates

Organism Group	Total of all Isolates Tested	Essential Agreement of Total	% Essential Agreement of Total
Streptococcus pneumoniae	152	147	96.7%
Streptococcus spp. Viridans Group	104	94	90.4%
Streptococcus spp. Beta-Hemolytic Group	202	195	96.5%
Total	**458**	**436**	**95.2%**

Challenge Isolates

Organism Group	Total of all Isolates Tested	Essential Agreement of Total	% Essential Agreement of Total
Streptococcus pneumoniae	25	24	96.0%
Streptococcus spp. Viridans Group	25	22	88.0%
Streptococcus spp. Beta-Hemolytic Group	25	24	96.0%
Total	**75**	**70**	**93.3%**

Total Isolates

Organism Group	Total of all Isolates Tested	Essential Agreement of Total	% Essential Agreement of Total
Streptococcus pneumoniae	177	171	96.4%
Streptococcus spp. Viridans Group	129	116	89.2%
Streptococcus spp. Beta-Hemolytic Group	227	219	96.3%
Total	**533**	**506**	**94.9%**

**Streptococcus* spp. Beta-hemolytic Group was tested with Mueller Hinton Broth supplemented with 2.5% Lysed Horse Blood

Summary Data and % Essential Agreement of Gram-Positive Clinical and Challenge Isolates using the Manual Read Method

Clinical Isolates

Organism Group	Total of all Isolates Tested	Essential Agreement of Total	% Essential Agreement of Total
Coagulase Negative *Staphylococcus*	93	93	100.0%
Staphylococcus aureus	135	135	100.0%
Enterococcus spp.	91	90	98.9%
Streptococcus spp. Beta-Hemolytic Group	75	75	100.0%
Total	**394**	**393**	**99.7%**

Challenge Isolates

Organism Group	Total of all Isolates Tested	Essential Agreement of Total	% Essential Agreement of Total
Coagulase Negative *Staphylococcus*	19	19	100.0%
Staphylococcus aureus	27	27	100.0%
Enterococcus spp.	12	12	100.0%
Streptococcus spp. Beta-Hemolytic Group	17	17	100.0%
Total	**75**	**75**	**100.0%**

Total Isolates

Organism Group	Total of all Isolates Tested	Essential Agreement of Total	% Essential Agreement of Total
Coagulase Negative *Staphylococcus*	112	112	100.0%
Staphylococcus aureus	162	162	100.0%
Enterococcus spp.	103	102	99.5%
Streptococcus spp. Beta-Hemolytic Group	92	92	100.0%
Total	**469**	**468**	**99.8%**

**Streptococcus* spp. Beta-hemolytic Group was tested with Mueller Hinton Broth supplemented with 2.5% Lysed Horse Blood

RESULTS cont.

Summary Data and % Essential Agreement of Gram-Positive Clinical and Challenge Isolates using the Auto Read Method

Clinical Isolates

Organism Group	Total of all Isolates Tested	Essential Agreement of Total	% Essential Agreement of Total
Coagulase Negative *Staphylococcus*	92	89	96.7%
Staphylococcus aureus	135	134	99.3%
Enterococcus spp.	91	84	92.3%
Streptococcus spp. Beta-Hemolytic Group	75	75	100.0%
Total	**393**	**382**	**97.2%**

Challenge Isolates

Organism Group	Total of all Isolates Tested	Essential Agreement of Total	% Essential Agreement of Total
Coagulase Negative *Staphylococcus*	19	19	100.0%
Staphylococcus aureus	27	27	100.0%
Enterococcus spp.	12	12	100.0%
Streptococcus spp. Beta-Hemolytic Group	17	17	100.0%
Total	**75**	**75**	**100.0%**

Total Isolates

Organism Group	Total of all Isolates Tested	Essential Agreement of Total	% Essential Agreement of Total
Coagulase Negative *Staphylococcus*	111	108	98.4%
Staphylococcus aureus	162	161	99.7%
Enterococcus spp.	103	96	96.2%
Streptococcus spp. Beta-Hemolytic Group	92	92	100.0%
Total	**468**	**457**	**97.6%**

**Streptococcus* spp. Beta-hemolytic Group was tested with Mueller Hinton Broth

Interlaboratory Reproducibility % Essential Agreement +/- one $\log_2$ Dilution of the Modal MIC

	Auto Read Gram Positive	Manual Read Gram Positive	Auto Read *Streptococcus* spp.	Manual Read *Streptococcus* s
Between-site total isolates tested (n)	75	75	75	75
Between-site isolates within +/- 1 well from mode (n)	73	74	72	72
Between-site reproducibility ratio	73/75	74/75	72/75	72/75
Between-site reproducibility %	97%	99%	96%	96
Total Essential Agreement (n)	**72**	**74**	**70**	**70**
Essential Agreement %	**96%**	**99%**	**93%**	**93%**

CONCLUSION

The Sensititre® 18 – 24 hour susceptibility system when compared to the CLSI M07 reference broth microdilution method demonstrated equivalent performance when testing **oritavancin** against gram-positive and fastidious clinical and challenge isolates.

The high level of essential agreement between the Sensititre® 18-24 hour susceptibility method and the CLSI reference method suggests that this is an acceptable method for susceptibility testing of **oritavancin**.

- **Clinical Isolates and CDC Challenge Organisms**
  Gram-positive Isolates:
  The overall essential agreement for **oritavancin** within +/- one $\log_2$ dilution, was 99.8% for the manual read method and 97.6% for the autoread method.
  Streptococcus spp. Isolates:
  The overall essential agreement for **oritavancin** within +/- one $\log_2$ dilution, was 96.8% for the manual read method and 94.9% for the autoread method.
- **Interlaboratory Reproducibility**
  Gram-positive Isolates:
  Reproducibility testing results for **oritavancin**, within +/- one $\log_2$ dilution from the Modal MIC result was, 97% for the autoread method and 99% for the manual read method.
  Streptococcus spp. Isolates:
  Reproducibility testing results for **oritavancin**, within +/- one $\log_2$ dilution from the Modal MIC result was, 96% for the autoread method and 96% for the manual read method.

REFERENCES

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